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IMPACT OF *CNIDOSCOLUS ACONITIFOLIUS* ON HEMATOLOGICAL PARAMETERS IN RATS

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Abstract

The plant *Chindoscolus aconitifolius* or often called Japanese papaya contains secondary metabolite compounds such as saponins, flavonoids, tannins, alkaloids, phytates, cyanogenic glycosides, and terpenoi. *C. aconitifolius* toxicity test on erythrocytes, leukocytes, and thrombocytes has never been done. The purpose of this study was to determine the effect *C. aconitifolius* leaves ethanol extract on erythrocytes, leukocytes, and thrombocytes in rats. Twhis research method uses experimental laboratory post-test. Tests on 24 female rats (*Rattus Norvegicus*) which were divided into 4 groups with 6 rats each. In the division of groups consisting of groups given 1% Na CMC (negative control), 50 mg/kg bw, 300 mg/kg bw and 2000 mg/kg bw carried out for 14 days. Statistically, the results showed no significant effect on the number of erythrocytes (p-value=0.338), leukocytes (p-value=0.750), and Thrombocytes (p-value=0.456). This study can be concluded that there is no toxic effect of *C. aconitifolius* Leaf Ethanol Extract on erythrocytes, leukocytes, and thrombocytes in rats (Rattus norvegicus).

Keywords: Chindoscolus aconitifolius, Erythrocytes, Leukocytes, Thrombocytes.

INTRODUCTION

Medicinal plants are plants that contain substances that can be used for therapeutic purposes and can serve as active ingredients or as precursors for the semisynthesis of new drugs, and it is estimated that 80% of most of the world's population uses them routinely to meet their primary health needs. A very important thing that must be done when using natural materials as medicinal raw materials is to know the toxicity effects of the compounds contained. (Jiménez-Arellanes *et al*, 2014). Japanese Papaya leaf (*Cnidoscolus aconitifolius*) is one type of plant that is cultivated as a vegetable, besides it has health benefits. *C. aconitifolius* has various pharmacological activities as a source of antioxidants, antidiabetic, antimultagenic, hypoglycemic, anti-inflammatory, antiprotozoal and antibacterial

(Nadiroh & Hariani, 2021). Not many toxicity studies of *C. aconitifolius* extracts have been conducted.

Toxicity tests allow researchers to identify toxic effects that may arise within a short time after administration of the substance. This includes the identification of target organs that may be affected. Oral acute toxicity tests help determine the degree to which a substance is harmful if ingested by humans. This information is important for setting safe doses and avoiding harmful side effects. One method of oral acute toxicity testing is using the OECD Guidelines. The OECD provides structured protocols (TG 420, 423, 425) for acute oral toxicity testing, focusing on lethal dose and apparent toxicity, which helps classify chemicals according to their hazard potential (OECD guidelines, 2001).

Acute oral toxicity tests are essential for assessing the safety of substances, with blood parameters serving as key indicators of toxicity. Various studies have explored different methodologies and different substances, revealing significant insights into their effects on blood parameters. This study aimed to look at the toxic effects of *C. aconitifolius* extract on several blood parameters namely, erythrocytes, leukocytes, and thrombocytes.

METHODS

This study is a laboratory experiment with a research design that is post-test only with control group design where the treatment effect is evaluated by comparing normal values and values after the effects of C. aconitifolius. The test animals were divided into 4 groups, namely Negative Control (Group A), 50 mg/kg bw (Group B), 300 mg/kg bw (Group C), 2000 mg/kg bw (Group D). Rats were placed in separate cages for two weeks for acclimatization under laboratory conditions (humidity 50% ± 10%, temperature 22°C ± 1°C, and under 12 h light - 12 h dark). Animals receive sufficient standard pellet food and drink water *ad libitum* according to standard procedures based on the Guidelines for the Care and Use of Laboratory Animals. All activities are carried out to minimize pain to the animals and utilize the animals as efficiently as possible. This research has been approved by the Ethical Committee of Bengkulu Polytechnic Health Research with Ethical Clearance certificate number No.KEPK.BKL/H/019/02/2024.

Extraction of C. Aconitifolius

The ethanol extract of *C. aconitifolius* was prepared using maceration method. *C. aconitifolius* was collected from several locations in Bengkulu Province, Indonesia. Dry simplisia of 500 grams of *C. aconitifolius* leaves were macerated using 70% ethanol solvent, then the maceration results were thickened using a rotary evaporator.

Blood sampling

For blood sampling, rats were anesthetized. Blood was drawn through the retro-orbitalis of the eye using a capillary pipette. Application can be done by puncturing the pipette with an angle of 45°. Blood was taken as much as 2 ml and then transferred into a vacuum tube containing EDTA anticoagulant.

Data Analysis

Data collected were several blood parameters, erythrocytes, leukocytes, and thrombocytes. The examination data were compared with the normal values of rat blood parameters. All data are mean ± SD, and differences between groups were determined using one-way analysis of variance (ANOVA), and further evaluated with Duncan's post hoc test. Data differences are considered significant if $p<0.05$.

RESULTS AND DISCUSSION

In this study, the body weight of rat test animals was measured every day to determine the difference in body weight of rats before and after giving ethanol extract of *C. aconitifolius* leaves (Table 1).

Table 1: The difference in body weight of rats before and after giving ethanol extract of C. aconitifolius leaves

	Before Treatment (Mean ± SD)	After Treatment (Mean ± SD)	*% Reduction*	*P-Value*
Group A	150,673 ± 22,691	144,871 ± 70,503	3,85%	0,235
Group B	152,706 ± 20,298	152,029 ± 70,830	0,44%	
Group C	178,205 ± 40,953	174,397 ± 84,442	2,14%	
Group D	159,613 ± 24,686	158,983 ± 66,879	0,39%	

Based on Table 1, there is a change in body weight in the test group before and after treatment with *C. aconitifolius* extract in rats. *C. aconitifolius* extract exerts varying effects depending on the dose. However, statistically the observed decrease in body weight of the rats was not significant. This decrease may be due to stress, environmental factors, or other factors not affected by the direct treatment. *C. aconitifolius* is known to contain various bioactive compounds, including flavonoids and antioxidants, which could affect the metabolism and energy balance of rats. Administration of *C. aconitifolius* extract can reduce cholesterol levels (Maghfiroh et al., 2021). Previous studies have

shown that extracts from this plant can affect the metabolism of rats, either directly through its effect on digestive enzymes or indirectly through its effect on the endocrine system (Orwa, 2009).

The administration of *C. aconitifolius* leaves extract was carried out for 14 days. Then the observation of erythrocytes, leukocytes, and thrombocytes in rats on the 15th day. The results of the examination of these blood parameters showed no difference. *C. aconitifolius* (Chaya) extract has various bioactive compounds such as flavonoids, saponins, polyphenols, and antioxidants that have the potential to affect the hematopoiesis (blood cell formation) system in the body.

Research shows that extracts from this plant can improve hematological parameters, especially in diabetic models. In a study involving alloxan-induced diabetic rats, administration of C. aconitifolius extract resulted in a significant increase in packed cell volume (PCV), red blood cells (RBC), hemoglobin (Hb), and white blood cells (WBC) (Azeez et al., 2010). The extract also improved platelet values and reduced erythrocyte osmotic fragility, indicating enhanced blood cell stability and function (Obichi et al., 2015)

Table 2: Erythrocyte count after administration of C. aconitifolius leaves ethanol extract

	Rats	**Erythrocytes**	**Mean ± SD**	***P-value***
Group A	1	NA	7,44 10^6/µL ± 1,06	0,338
	2	NA		
	3	NA		
	4	8,2 10^6/µL		
	5	6,69 10^6/µL		
Group B	1	7,38 10^6/µL	7,50 10^6/µL ± 0,40	
	2	7,06 10^6/µL		
	3	NA		
	4	8,02 10^6/ µL		
	5	7,57 10^6/µL		
Group C	1	NA	7,10 10^6/µL ± 0,05	
	2	NA		
	3	7,06 10^6/µL		
	4	NA		
	5	7,14 10^6/µL		
Group D	1	NA	6,96 10^6/µL ± 1,61	
	2	6,67 10^6/µL		
	3	NA		
	4	7,12 10^6/µL		
	5	7,11 10^6/µL		

PS: Normal range of erythrocytes: 7,0-9,0 x 10^6/µL (Weiss & Wardrop, 2011)

Erythrocytes or red blood cells play a role in carrying oxygen to all body tissues. The number of erythrocytes in the blood is strongly influenced by health status, tissue oxygenation, and antioxidant levels in the body. *C. aconitifolius* extract is rich in iron and antioxidants that are important in the process of erythropoiesis (red blood cell production) (Camaschella et al., 2022). Iron is necessary for hemoglobin formation, while antioxidants such as flavonoids help protect erythrocyte precursor cells from oxidative damage in the bone marrow (Kim & Nemeth, 2015).

Oxidative stress can damage red blood cells and shorten their lifespan. Antioxidants in *C. aconitifolius* extract such as vitamin C and flavonoids serve to protect the erythrocyte membrane from damage by free radicals, thereby extending the life of erythrocytes and maintaining their number in blood circulation. The iron content and antioxidants in *C. aconitifolius* support good hemoglobin formation, so it can help in anemia conditions (de Vasconcellos et al., 2023). If hemoglobin levels are good, the body is able to maintain the number of erythrocytes at an optimal level. C. aconitifolius extract can increase erythrocyte production and protect red blood cells from oxidative damage.

Table 3: Leucocyte count after administration of C. aconitifolius leaves ethanol extract

	Rats	**Leucocyte**	**Mean ± SD**	***P-value***
Group A	1	NA	10,05 10^3/µL ± 3,33	0,750
	2	NA		
	3	NA		
	4	12,41 10^3/µL		
	5	7,7 10^3/µL		
Group B	1	7,41 10^3/µL	9,08 10^3/µL ± 2,73	
	2	12,9 10^3/µL		
	3	NA		
	4	9,19 10^3/µL		
	5	6,82 10^3/µL		
Group C	1	NA	9,52 10^3/µL ± 1,03	
	2	NA		
	3	8,79 10^3/µL		
	4	NA		
	5	10,25 10^3/µL		
Group D	1	NA	9,49 10^3/µL ± 6,63	
	2	16,95 10^3/µL		
	3	NA		
	4	4,23 10^3/µL		
	5	7,29 10^3/µL		

PS: Normal range of Leucocyte: 6,0–18,0 x 10^3/µL (Weiss & Wardrop, 2011).

Leukocytes or white blood cells are responsible for maintaining the body's immunity. Leukocyte counts are influenced by the body's response to infection, inflammation, or oxidative stress. *C. aconitifolius* extract has anti-inflammatory properties that may affect leukocyte production and regulation (Padilla-Camberos et al., 2021). The flavonoids and phenolic compounds in this extract can reduce the production of pro-inflammatory cytokines (such as TNF-α, IL-6), which in turn can lower the number of leukocytes produced in response to chronic inflammation (Babalola et al., 2021). In other words, this extract can help maintain the balance of leukocytes in the body and prevent excessive inflammatory responses.

The strong antioxidant content can strengthen the immune system by increasing the activity of leukocytes, especially lymphocytes and macrophages, in the face of pathogens. This extract may help boost the body's immune response by stimulating phagocytosis (the process by which leukocytes eat pathogens). In conditions of infection or stress, leukocyte counts often increase as the body's immune response. *C. aconitifolius* has potential as an immunomodulatory agent, which can stimulate leukocyte production when needed, such as in bacterial or viral infections, while suppressing overproduction when unwanted inflammation occurs. Furthermore, the immunomodulatory effects of C. aconitifolius were highlighted, indicating its potential to enhance immune responses in infected models (Hidayati et al., 2024).

C. aconitifolius extract can act as an immunomodulator, which can increase or decrease the number of leukocytes according to the needs of the body, through anti-inflammatory mechanisms and enhancing the immune response.

Table 4: Thrombocytes count after administration of C. aconitifolius leaves ethanol extract

	Rats	**Thrombocytes**	**Mean ± SD**	***P-value***
Group A	1	NA	741 x 10^3/µL ± 2,82	
	2	NA		
	3	NA		
	4	739 x 10^3/µL		
	5	743 x 10^3/µL		
Group B	1	697 x 10^3/µL	707 x 10^3/µL ± 116,87	0,456
	2	847 x 10^3/µL		
	3	NA		
	4	723 x 10^3/µL		
	5	562 x 10^3/µL		
Group C	1	NA	903 x 10^3/µL ± 94,75	
	2	NA		
	3	970 x 10^3/µL		
	4	NA		
	5	836 x 10^3/µL		

Group D	1	NA	734,66 x10^3/µL ± 163,59
	2	617 x 10^3/µL	
	3	NA	
	4	621 x 10^3/µL	
	5	966 x 10^3/µL	

PS: Normal range of Thrombocytes: 500–1000 x 10^3/µL (Weiss & Wardrop, 2011).

Thrombocytes function in the process of blood clotting and repair of injured tissues. The number of thrombocytes in the blood is affected by inflammatory conditions, tissue damage, and the body's ability to produce thrombocytes in the bone marrow. *C. aconitifolius* extract contains essential nutrients that can support thrombopoiesis (Obichi et al., 2015). Antioxidants and essential nutrients such as iron, folic acid, and vitamin K from this extract may help increase thrombocytes production in the body, especially in conditions of thrombocytes deficiency (thrombocytopenia).

Oxidative stress can damage thrombocytes and shorten their lifespan. The powerful antioxidants in this extract can protect thrombocytes from oxidative damage, thus helping to maintain adequate thrombocytes counts in the blood. *C. aconitifolius* is also known to contain vitamin K, which plays a role in the coagulation (blood clotting) process (Victor et al., 2016). This helps in maintaining sufficiently active thrombocytes to support the blood clotting process when a wound or injury occurs. *C. aconitifolius* extract can support platelet production and protect platelets from damage, while improving coagulation function through vitamin K content.

CONCLUSION

The conclusion of the study is that the ethanol extract of *Chindoscolus aconitifolius* leaves at doses of 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight administered for 14 days had no statistically significant effect on the number of erythrocytes, leukocytes, and thrombocytes in rats. Therefore, the extract did not exhibit toxicity in these blood parameters within the conditions of the study.

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